Sigeru DAIGOBO*:　**Observations on the embryos and young sporophytes of** *Botrychium multifidum*
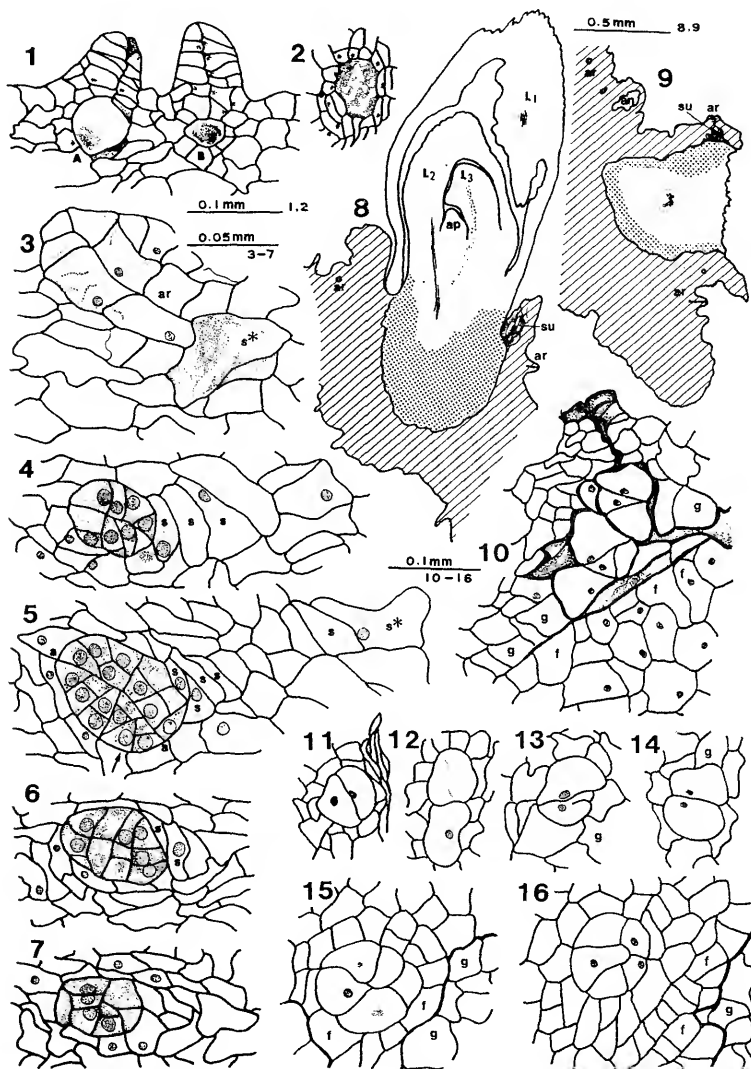
大悟法　滋*:　ヤマハナワラビの胚と若い胞子体

The embryogeny of *Botrychium* varies greatly from one species to another according to the studies of *B. virginianum* (Jeffrey 1897, Campbell 1911), *B. obliquum* (Lyon 1905, Campbell 1921), *B. lunaria* (Bruchmann 1906) and *B. simplex* (Campbell 1922). The growth form of the zygote before division, the presence or absence of a suspensor, the type of polarity during embryo development, the juxtaposition of embryonic organs to each other, the degree of foot development, and the relative time of appearance of each organ are all variable characteristics of the *Botrychium* embryo. Lyon (1905) emphasized the presence of a suspensor in the embryo of *B. obliquum*, and established the genus *Sceptridium* based on it. He transferred all the *Botrychium* species of the ternate type recognized by Plantl (1884). However, many workers did not agree that the suspensor was an appropriate diagnostic feature of the genus *Sceptridium*, because it had been verified only in *B. obliquum*. Nishida (1952b) accepted the genus *Sceptridium* not because of the presence of suspensor but based on the vascular anatomy of the phyllomorphore. Later he (1955) also described a suspensor for the ternate members of *Botrychium*. Anatomical studies on the adult sporophyte of *Botrychium* were carried out by Chrysler (1910, 1945), Campbell (1911), Lang (1913), Nozu (1950), Nishida (1952a), Kato (1978) and many others. Details of the anatomy of the young sporophyte of the ternate group have been studied in only a few species.

The gametophytes accompanying young sporophytes of *B. multifidum* were described previously (Daigobo 1979). As part of a continuing study the anatomical features of the embryo and young sporophyte of *B. multifidum, Sceptridium* sensu Lyon (1905), are described in this report.

Materials and methods　*B. multifidum* was collected at Sugadaira, Nagano Prefecture, Japan, as previously described (Daigobo 1979). About 30 gametophytes and 40 young sporophytes of *B. multifidum* were studied. Gametophytes

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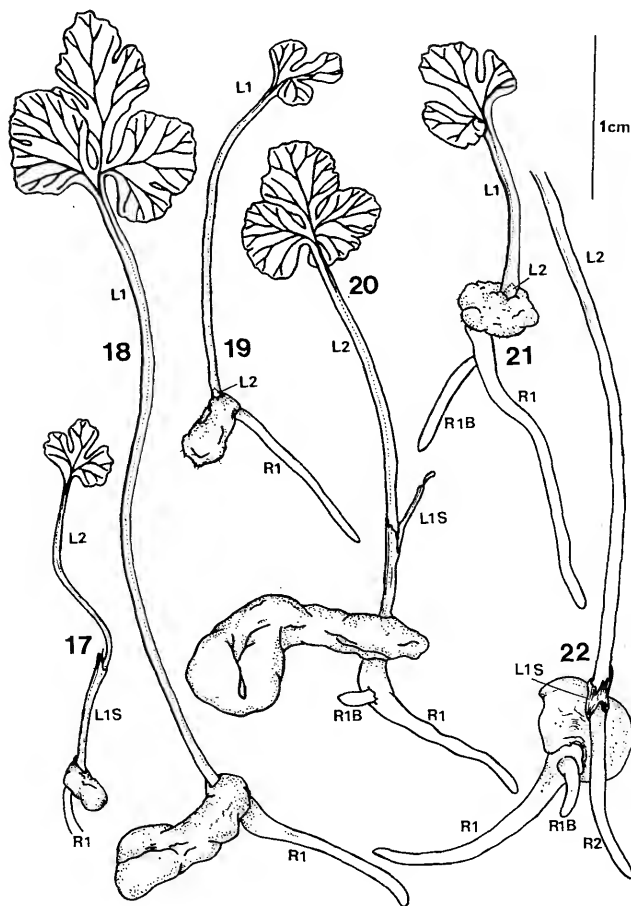
Figs. 1-16. Embryogeny of *B. multifidum*. 1. Longitudinal section of post mature (A) and mature (B) archegonia. 2. Cross section through the venter region of an old post mature archegonium. 3-7. Five of a series of sections of a young embryo. Suspensor cells (s), archegonia (ar), the first partition wall of the embryo (a-a), and the position of archegonial base (s*). 8. Longitudinal section of a young sporophyte. Suspensor (su), archegonia (ar), first to third leaves (L1-L3), shoot apex (ap), foot (stippled), and the tissue of gametophyte (shaded). 9. Transverse section of boundary between shoot and root in the same stage as Fig. 8. Suspensor (su), archegonia (ar), antheridium (an), foot (stippled), and tissue of gametophyte (shaded). 10. A longitudinal section of the suspensor, more highly magnified of Fig. 9-su. Tissue of foot (f) and gametophyte (g). 11-16. Six of a series of cross sections of a suspensor in the same stage as Figs. 8 and 9. Tissue of foot (f) and gameto-phyte (g).

2-3 mm in length were employed for observation of young embryo. The young sporophytes were studied while still attached to the gametophytes. All organisms were fixed in CRAF I solution or FAA solution, and then embedded in paraffin for serial sectioning. Two combinations of fast green with Heidenhain's hematoxylin or safranin were used to stain the sections.

Observations and discussion Archegonia of *B. multifidum* were born along the antheridial ridge on the dorsal surface of the gametophyte. Old archegonia with open terminal neck cells on the mature gametophytes were used to observe embryogeny. Initially egg cells showed an increase in the size (Figs. 1, 2). Although fertilization was not directly observed, the enlarged cell is apparently a unicellular embryo before the first division as described in other *Botrychium* species. The unicellular embryo is not so elongate nor curved as that of *B. obliquum* (Campbell 1921) and *B. japonicum* (Nozu 1954). Although it is common that more than one egg is fertilized in the same gametophyte, only one sporophyte developed on a gametophyte.

The young embryo maintained a broadly oval outline (Figs. 3-7). Fig. 5 illustrates the wall (a-a) which is the first division plane of an embryo sectioned slightly obliquely in a nearly median plane. The triangular cell (arrow) of the epibasal tier appears to be the shoot initial, although no progeny cells were observed to confirm this. The suspensor is composed of several cells, and is slightly curved, embedded within the tissue of gametophyte (Figs. 5-7). They are recognizable because they are connected with the hypobasal tier of the embryo and the basal part of the archegonium (s*). The suspensor is maintained throughout the later stages of embryo development (Figs. 8, 9). Fig. 10, an enlargement of the longitudinal section of Fig. 9, and Figs. 11-16 are diagrams of a series of transverse sections of different suspensor in the same stage. The suspensors were also composed of several cells at this stage, and had the same structure as in the young embryo. Lyon (1905) illustrated a single suspensor cell attached to the young sporophyte of *B. obliquum*. However, the adjacent cells were undoubtedly also suspensor cells as found in *B. multifidum*. Campbell (1921) reported large undivided suspensor cells in young embryos of *B. obliquum*. Nishida (1955) demonstrated a multicellular suspensor at an early embryonic stage in *B. japonicum*, more massive than that of *B. multifidum*.
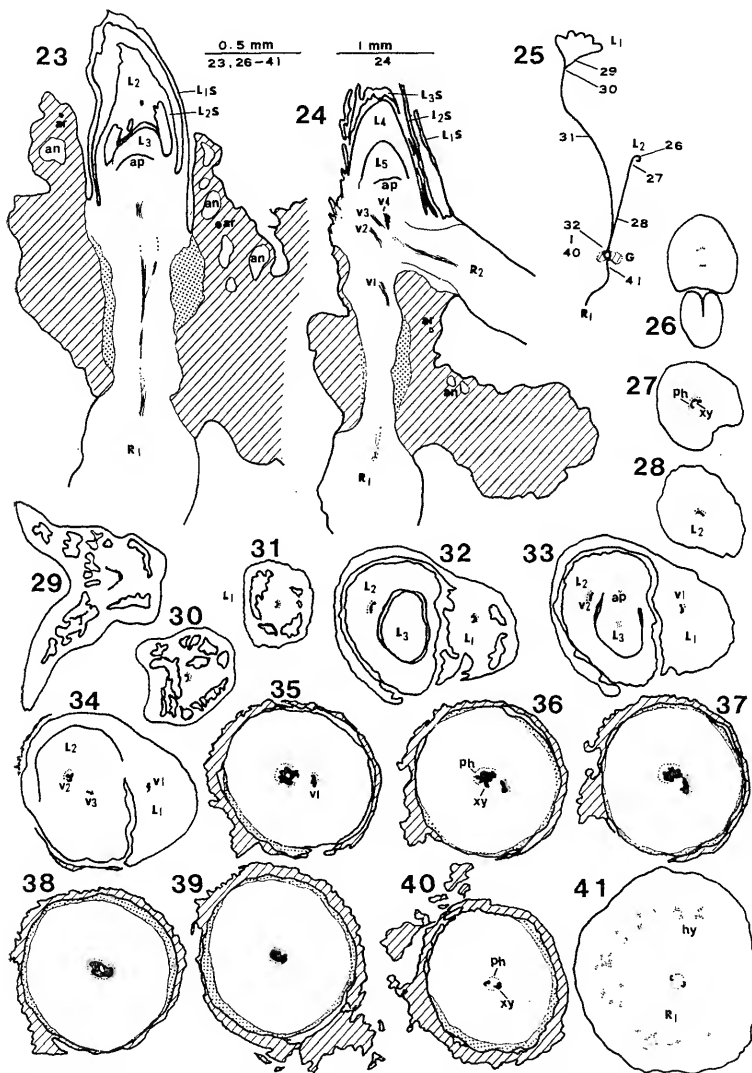
Young sporophytes attached to the gametophytes possess the first roots,

Figs. 17-22. Young sporophytes attached to gametophytes. First and second leaves (L1, L2), stiplar sheaths of first leaves (L1S), first and second roots (R1, R2), and lateral roots from first roots (R1B).

rarely second roots, and the first or second leaves (Figs. 17-22). In many cases, the 2nd-5th leaves were already developed in the base of older leaves (Figs. 19, 21, 23, 24).

The first roots are usually about 10 mm in length and reached 15-20 mm in several cases. In all cases examined, the first roots emerged from the lower

Figs. 23–41. Young sporophytes. 23–24. Longitudinal sections of young sporophytes. Second to fifth leaves (L2–L5), stiplar sheath of the first to third leaves (L1S–L3S), shoot apex (ap), first and second roots (R1, R2), vascular bundles to the leaves (V1–V4), archegonia (ar), antheridia (an), tissues of foot (stippled) and gametophytes (shaded). 25. Diagram of the levels of the sections, Figs. 26–41. The first leaf is still intact. 26–41. A series of transverse sections showing the progressive changes in the stele and its position from leaf to root. The first to third leaves (L1–L3), first root (R1), shoot apex (ap), xylem (xy), phloem (ph), foot (stippled), gametophyte (shaded), and hyphae (hy).

surface of the gametophyte. Short lateral roots had also developed off the main roots (Figs. 20–22). Campbell (1921) suggested that the lateral roots were probably the result of injury to the apex of the primary root. However, it is a normal elaborate branching pattern that characterizes the root system of *B. multifidum*, according to my observations.
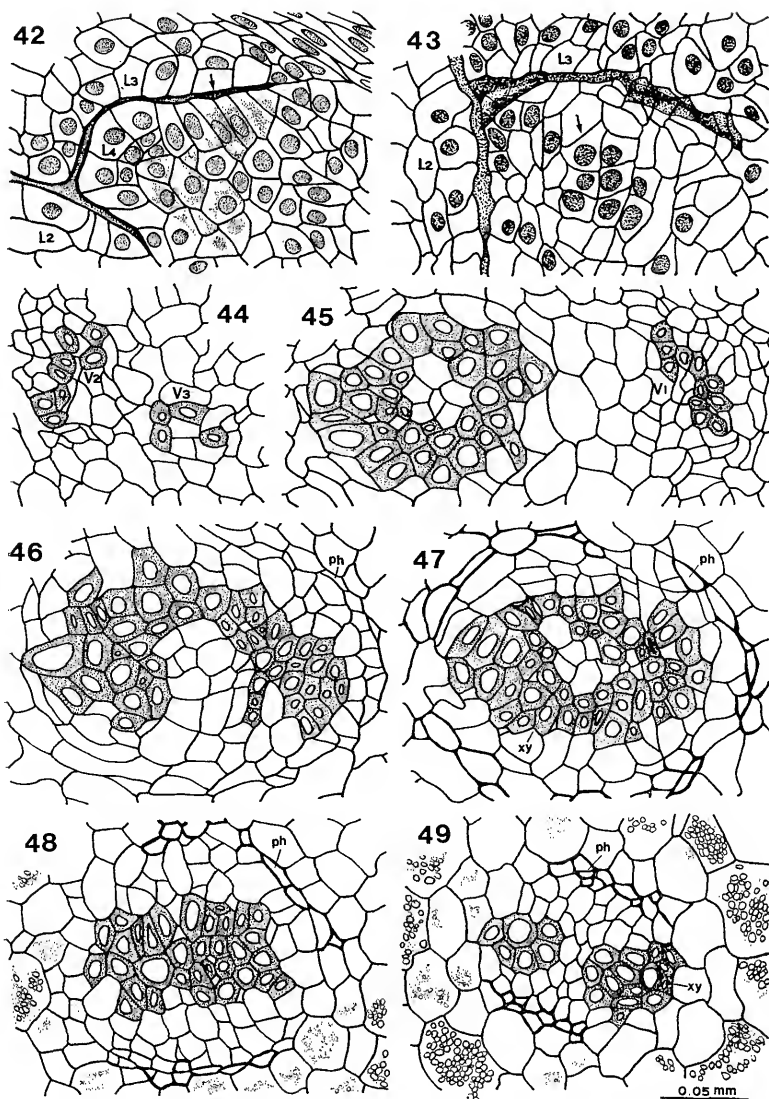
Campbell (1921) described that in *B. obliquum* the foot became unrecognizable as the root grew. Bierhorst (1971) mentioned that the foot of the same species did not appear as a protrusion but as a half-girdle cell mass with the characteristic contents and staining reactions of foot tissue. In *B. multifidum* the foot is also recognizable at the junction between the leaf and root. The well developed foot surrounds the tissue of the under part of shoot, and seems to be penetrated by the first root (Figs. 23, 24, 35–40).

The development of the vascular bundle of first root began probably at a very early stage just as in *B. obliquum* (Bass-Becking 1921). There was already an elaborate vascular system in the first roots observed in this study. The bundle is diarch, and the endodermis relatively inconspicuous (Fig. 49).

The cortex layer which grew out from the tissue of the gametophyte was always infected by a fungus (Figs, 44, 53), however, the foot and upper part of root which were embedded in the tissue of gametophyte never contained fungal hyphae.

The second root of *B. multifidum* developed much later at the time when the fourth or fifth leaves were developing (Fig. 24). It originated from the upper side of the gametophyte, and broke through the sheaths of first and second leaves at the base of the shoot (Fig. 22). The vascular system of the second root is, like the first, diarch and seems to connect to the bundle of the third or fourth leaves, not to the second leaf as in *B. obliquum* (Bass-Becking 1921). The contractile root which is characteristic of the older root of *B. multifidum* (Stevenson 1975) was not observed in the first or second root.

The first leaf was usually about 20 mm in length, but rarely reached 50 mm. The size of the leaf seems to depend mainly on the size of gametophyte and its depth in the soil. The first leaf emerged from the dorsal side of the gametophyte, and appeared above ground as a ternate lamina with a long slender petiole (Figs. 18, 19, 21). The petiole of the mature first leaf had many lacunae in the cortex (Figs. 30, 31, 50). The intercellular spaces increase at the base of petiole. The base became the stiplar sheath which surrounded
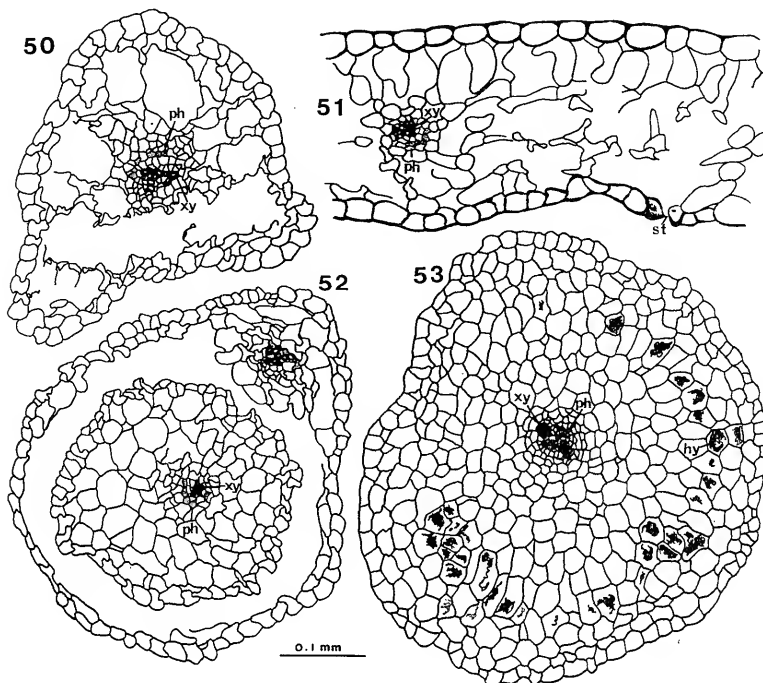
Figs. 42–49. Young sporophytes. 42. A longitudinal section of the shoot apex, more highly magnified of Fig. 8-ap. 43. A transversal section of the shoot apex, same stage of the sporophyte in Figs. 8 and 42. 44–49. Progressive change of the stele, more highly magnified of the series shown Figs. 34–41. Showing the vascular bundles to the first to third leaf (V1–V3), xylem (xy), phloem (ph).

second leaf (Figs. 32-34, 52).   The sheath broke at the base and stained dark brown until much later (Figs. 17, 20, 22-24).

The stomata (st) usually develop on the ventral and rarely dorsal side of lamina (Fig. 51).   The second leaf was the same in size and structure as the first leaf except the base was enveloped by the sheath from first leaf stipe. Longitudinal (Fig. 42) and transversal (Fig. 43) sections of the shoot apex show the dividing apical cell (arrow) and the groups of cells of the fourth (L 4) leaf primordia.

Serial transverse sections (Figs. 26-41) of the young sporophyte revealed the progressive change in the stele and its position from leave to root.   The base of the stipe (Fig. 32) showed the third leaf developing in the stiplar

Figs. 50-53. Cross sections of young sporophytes.   50. Upper portion of stipe, showing the under side is the adaxial side. Xylem (xy), phloem (ph).   51. A part of lamina of first leaf, showing a stoma (st).   52. Basal portion of the stipe, showing the stipe of second leaf is surrounded by the stiplar sheath of first leaf.   53. First root, showing the extent of fungus by the diagramatic hyphae (hy).

sheath of second leaf. A section through the shoot apex (Fig. 33) shows that the vascular tissue was initiated in the third leaf near the growing tip. Basally at the level of the surface of the gametophyte, several tracheids of third leaf were observed (Figs. 34, 44). More basally the two bandles of the second and third leaves approached each other and finally joined (Figs. 35, 45). The xylem of the two leaf traces of the first leaf and the conjugated bundle, also joined in the basal region (Figs. 36-38, 46-48). The stele divided again into two strands (Figs. 39, 40), and continued downward into the root (Figs. 41, 49). The xylem medullated in the conjugated bundle (Figs. 35, 38, 45, 47). The pith was simply an inclusion of the parenchymas between the root and leaf traces as in *B. obliquum* (Bass-Becking 1921) and not of infrastelar origin. The phloem cells were shrunken and appeared thick walled. They appeared on the abaxial side of the xylem in the leaf trace, and tended to surround the xylem where the xylem conjugated. In the root, the phloem divided into two strands at right angles to the xylem (Figs. 41, 53).

As mentioned above, the anatomical features of embryos and young sporophytes are essentially same as in *B. obliquum* (Lyon 1905, Campbell 1921, Bass-Becking 1921), except that the unicellular embryo is not so elongate, the suspensor is composed of several cells in the early embryonic stage, the foot is more developed, and the second root appears later.

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　ヤマハナワラビの胚と若い胞子体の解剖学的な観察を行った。胚の形態は *B. obliquum* (Lyon 1905, Campbell 1921) やオオハナワラビ (西田 1952b) によく似ているが，受精後の卵細胞が増大しても，それほど伸長しない点が異なる。本種においても胚柄の存在が確認され，胚の比較的若い時期から多数の細胞からなり，第3葉が形成され始める頃まで同様の形態が観察された。この点はオオハナワラビの胚柄とよく似ているが，オオハナワラビのように塊状にはならず，構成する細胞数はずっと少なく，胚との接着点を除いて細胞が1〜2列に連なって見える。若い胞子体の内部形態も *B. obliquum* (Bass-Becking 1921) と基本的に同じ形態が観察されたが，若い胞子体の葉と根の移行部をよく発達した foot がとりまくこと，2番目の根の発達がずっと遅いことなどの点で違いが見られた。